

Structural Basis of Activation of WNK Kinases by Hydrostatic Pressure E. J. Goldsmith*, Radha Akella, and Jenny Jiou
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WNK1 is a protein kinase on pathway for the regulation of cation-chloride cotransporters (CCCs), important mediators of transepithelial transport and cell volume control. CCCs are regulated by chloride and cell-external osmolarity in a phosphorylation dependent manner. We demonstrated recently that the ability of WNK1 to autophosphorylate is inhibited by chloride, and that the kinase domain of WNK1 directly binds chloride ion. The phosphorylated form of the kinase domain of WNK1 (210-483)/S* adopts a different configuration in the activation loop, closer to a fully active kinase, revealing the conformational equilibrium nature of the chloride inhibition.

The chloride inhibition of WNK1 prompted us to ask whether WNK1 is activated by osmotic or hydrostatic pressure, as anticipated from known regulatory mechanisms of CCCs. Using macromolecular crowding surrogates such as polyethylene glycols we found that it is indeed activated by polyethylene glycol, and that this regulation is opposed by chloride. Similarly, WNKs 1 and 3 are activated by hydrostatic pressure applied with multiple modalities. The structural basis of this regulation is proposed based on the kinase domain of inactive, chloride bound WNK1.